TOROIVD TECHNOLOGY COMPANY LIMITED.
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TOROIVD® 5G qPCR Premix Screening Kit

【Catalogue Number】 QPT-204U

【Packing Information】 1.25 mL/tubes ×4 types

【Description】

TOROIVD® 5G qPCR Premix Screening Kit, cotainning 4 kind of 2×master mix, is specially designed for IVD reagent manufacturers to screening the optimal raw materials. 2×master mix contains the TOROIVD®5G DNA polymerase, Uracil-DNA Glycosylase, dNTPs, dUTP and reaction buffer, except for the primer and template DNA.All premixes are developed based on different customer demands,and widely used as raw materials for IVD reagent manufacturers.Furthermore, all premixes have followed the ISO13485 quality management system for large-scale production. With the excellent performance of high speed, high sensitivity, high stability, and inhibitor tolerant, all premixes contain Uracil-DNA Glycosylase/dUTP for removing the the crossed contamination caused by PCR product.

【Feature】

-Shorten R&D cycle

No need to optimize the reaction buffer, the optimal raw materials for qPCR can be quickly screened.

-Rapid and highly sensitive

With the optimal premix, 2-copies targets per reaction can be detected within 30 minutes only by optimizing the concentration of the primers and probes.

-Inhibitor tolerant

The unique proprietary formulation of the premixes allows robust performance even in the presence of PCR inhibitor, so direct qPCR with extraction-free can be successfully performed.

-Room-temperature stable:

The specially optimized PCR buffer make the premixes very stable at room temperature. Therefore, the performance is not easily decrease during storing and shipping.

-Avoid Contamination

This premixes contains dUTP and UNG in the reaction buffer . The crossed contamination caused by PCR product can be removed so that the rate of false-positive detection can be reduced.

【Application】

Development of IVD reagents based qPCR and RT-qPCR

【Components】

Components (Name/Cat No.)	Volume	Quantity
TOROIVD® 5G qPCR Premix with UNG (QPT-200U)	1.25 mL	1
TOROIVD® 5G qPCR Premix BB with UNG (QPT-200U-BB)	1.25 mL	1
TOROIVD® 5G qPCR Premix SS with UNG (QPT-200U-SS)	1.25mL	1
7G One® Premix with UNG (QPR-331)	1.25mL	1

【Primer/Probe Design】

-Design of primers

Primer length: 18–25bp； Tm of primer: 60–65°C； GC content: 40–60%； Target length: 70–200 bp;

Larger targets (>200 bp) tend to reduce the efficiency and specificity of amplification.

Purification grade: OPC or HPLC grade;

-Design of probes

Probe length: 20–30bp; Tm of probe: 65–70°C; GC content: 40–60%; Purification grade: HPLC.

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-Design for avoiding Contamination

Amplicon with high GC content will degrade the the performance of UNG for removing the contamination caused by PCR product. Therefore, when designing the primers, it is necessary to minimize the GC content of the amplicon as much as possible.

【Template Preparation】

1. Purified template DNA or RNA can be may be used directly or after dilution
2. For direct qPCR with extraction-free samples, the premix may be inhibited by some VTM or dilution buffer. It is recommended the VTM or dilution buffer based Tris buffer, such as TE buffer. The addition of surfactants (Triton X-100 or Tween-20)within 5% helps to lysis the sample and inactivate viruses.
3. Loading 10μL purified template or diluted sample per well in two 8-strip qPCR tubes.

Notes: *It is recommended to use approximately 100copies targets per reaction*

【Amplification of DNA】

1. Preparation of the reaction mix

-The 2× qPCR premixes should be fully thawed , gently vortexed and briefly centrifuged.

Notes: *Due to the high concentration stabilizer，there may be crystal precipitation in the premix ，which can be used normally after being fully thawed at room temperature.*

-Prepare the primers and probes for 4× primer&probe premix

Note: *The final concentration is recommended to 0.4 μM for the primes and 0.1 μM for the probes.*

-Prepare the following reaction mix for 5 reactions at total 40μL reaction volume as follows :

Component	5 reactions
2× qPCR Premix with UNG	100μL
4×Primer&Probe Premix	50μL
Total	150 μL

-Mix thoroughly by vortexing and centrifuge immediately.

-Loading 30 μL reaction mix per well in the qPCR tube 10μL template.

-Set four technical replicates for each premix.

-Gently vortexed and briefly centrifuged.

2. Set up the qPCR cycler:

-Select the corresponding fluorescence detection channel based on the fluorescence labeling of the probe.

-Set up the universal cycling conditions for all qPCR cyclers as follows:

Steps		Temperature	Time	Cycles
1	UNG enzyme action	37°C	2 min	1
2	Prenaturation	95℃	3min	1
3	Denaturation	95°C	15 sec	45
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

3. Result analysis

-Select the Select the optimal premix based on the Cq value and fluorescence signal value.

-Contact us to order the optimal premix for further development and optimization.

【Amplification of RNA】

1. Preparation of the 1-step RT-qPCR Premix

-The 2× qPCR premixes should be fully thawed , gently vortexed and briefly centrifuged.

Notes: *Due to the high concentration stabilizer，there may be crystal precipitation in the premix ，which can be used normally after being fully thawed at room temperature.*

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-Add RNase inhibitor and reverse transcriptase into 100μL premix, gently vortexed and briefly centrifuged.

Notes:

-The total volume of reverse transcriptase and RNase inhibitor reagents should not exceed 5μL.

-If using a frereeze-dried tube reverse with transcriptase and RNase Inhibitor from TOROIVD,100μL premix can be directly added into frereeze-dried tube.

2. Preparation of the reaction mix

-Prepare the primers and probes for 4×primer&probe premix

Note: *The final concentration is recommended to 0.4 μM for the primes and 0.1 μM for the probes.*

-Prepare the following reaction mix for 5 reactions at total 40μL reaction volume as follows :

Component	5 reactions
2×1-step RT-qPCR Premix	100μL
4×Primer&Probe Premix	50μL
Total	150 μL

-Mix thoroughly by vortexing and centrifuge immediately.

-Loading 30 μL reaction mix per well in the qPCR tube with 10μL template.

-Set four technical replicates for each premix.

-Gently vortexed and briefly centrifuged.

3. Set up the qPCR cycler:

-Select the corresponding fluorescence detection channel based on the fluorescence labeling of the probe.

-Set up the universal cycling conditions for all qPCR cyclers as follows:

Steps		Temperature	Time	Cycles
1	UNG enzyme action	37°C	2 min	1
2	Reverse transcription	50°C	10min	1
2	Prenaturation	95°C	3min	1
3	Denaturation	95°C	15 sec	45
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

4. Result analysis

-Select the Select the optimal premix based on the Cq value and fluorescence signal value.

-Contact us to order the optimal premix for further development and optimization.

【Storage】

This reagent can be stored at 2-8°C for 12 months.

For longer storage, this reagent should be kept at -20°C.

【Contact】

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